AN *IN VITRO* EVALUATION OF *MANGIFERA INDICA* GUM AS A POTENTIAL EXCIPIENT FOR ORAL CONTROLLED-RELEASE MATRIX TABLET.

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Running title: Formulation of Sustained Release Matrix Tablets of Lornoxicam

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Summary

The main aim of the present investigation was to develop matrix tablets of lornoxicam with *Mangifera indica* gum and to study its functionality as a matrix forming agent for once daily sustained release tablet formulations. Six batches of sustained release matrix tablets of lornoxicam were prepared by using different drug: polymer ratios viz.1:1, 1:1.5, 1:2, 1:2.5, 1:3, and 1:3.5 for *Mangifera indica* gum. *Mangifera indica* gum was used as matrix forming material, while microcrystalline cellulose was used as diluent. The tablets had uniform physical appearance, average weight, drug content, and adequate hardness. The results of in vitro release conducted using USP type II dissolution rate apparatus, in a dissolution media comprising of 900 mL of 0.1 N HCl for 2 h followed by phosphate buffer (pH 6.8) for 24 h at 37°C and 50 rpm, revealed that as the proportion of mucilage in the matrix was increased there was a corresponding decrease in the release of drug. A better sustained drug release (98%) was obtained with the matrix tablet (Batch F6) of the *Mangifera indica* gum. Swelling study was also carried out to study dispersibility of gums at different concentrations. The swelling studies revealed that, as the proportion of gum in tablets was increased, there was a corresponding increase in percent swelling and a decrease in percent erosion of tablets. The Differential Scanning Calorimetric (DSC) and Fourier Transform Infrared (FTIR) study revealed that there was no negative chemical interaction between drug and the gum used. Stability studies were conducted at 40±2ºC and RH 75±5% for 3 months indicates that lornoxicam was stable in the matrix tablets. It was concluded that dried *Mangifera indica* gum can be used as an excipient for making sustained release matrix tablets.

Keywords: *Mangifera indica* gum, lornoxicam, matrix tablets, sustained release, Pharmaceutical excipients.

Introduction

Oral sustained release systems continue to dominate the market despite the advancements made in other drug delivery systems in order to increase the clinical efficacy and patient compliance. From a practical pharmaceutical view point, numerous types of polymers are currently employed to control the drug release from the pharmaceutical dosage form. Oral sustained release systems are mainly grouped into three types, e.g. reservoir, monolithic and matrix types[1-2]. Among these hydrophilic matrix tablets are preferred in the formulations since most display good compression characteristics, even when directly compressed and have adequate swelling properties that lead to a rapid formation of external layer, allowing drug release modification.

In recent years, plant derived polymers have mucilages can occur in high concentrations in different evoked tremendous interest due to their diverse pharmaceutical applications such as diluent, binder, disintegrant in tablets, thickeners in oral liquids, protective colloids in suspensions, gelling agents in gels and bases in suppository, they are also used in cosmetics, textiles, paints and paper-making. These polymers such as natural gums and mucilage are biocompatible, cheap and easily available and are preferred to semi synthetic and synthetic excipients because of their lack of toxicity, low cost, availability, soothing action and non irritant nature. Demand for these substances is increasing and new sources are being developed. India, because of its geographical and environmental position, has traditionally been a good source for such products among the Asian countries. Still, large quantities are imported from Europe to meet increasing demand. Regular research is going on in field of use of natural occurring biocompatible polymeric material in designing of dosage form for oral controlled release administration. Natural gums are biodegradable and nontoxic, which hydrate and swell on contact with aqueous media, and these have been used for the preparation of dosage form[3]. Pectins, including high and low ester and amidated, are used in food all over the world. It is an edible plant polysaccharide, has been shown to be useful for the targeted drug delivery systems for specific class of drugs such as proton pump inhibitors and anticancerous drugs.

Tamarind gum and pectin are hydrophilic polymers, which until recently had been limited for use in gelation, thickening, suspending and emulsifying water based systems[4-6].

For centuries, the Mango tree (Scientific name: *Mangifera indica*, Family: Anacardiaceae) has been an integral part of life in India. Each and every part of the tree (bark, leaves, root and kernel seed fruit) serves a certain purpose, for instance, as diuretic, astringent, aphthous stomatitis, diabetes, asthma, diarrhea, urethritis, dysentery, scabies and other parasitic skin diseases[7].

Lornoxicam, also known as chlortenoxicam[8], is a member of the oxicam group of nonsteroidal anti-inflammatory drugs (NSAIDs) with extremely potent anti-inflammatory and analgesic activities. Lornoxicam is commercially available in the form of conventional immediate-release tablets (4 and 8 mg), rapid-release tablets (8 mg), and parenteral formulations (4 mg/ml) for intravenous and intramuscular use. It is widely used for the symptomatic treatment of pain and inflammation in patients with rheumatoid arthritis and osteoarthritis[9]. Moreover, it showed great efficacy in various clinical trials in the management of perioperative and postoperative pain associated with gynecological, orthopedic, abdominal, and dental surgeries. However, lornoxicam's usefulness is limited due to its short half-life that ranges from 3 to 5 h[10-11]. Added to that, lornoxicam shows a distinct pH-dependent solubility characterized by very poor solubility in acidic conditions present in the stomach. Thus, it remains in contact with the stomach wall for a long period which might lead to local irritation and ulceration[12]. During earlier study in our laboratory, the disintegrating properties of *Mangifera indica* gum were evaluated[13]. Literature survey reveals that comprehensive physicochemical characterization and pharmaceutical application of the Mangifera indica gum (MIG) as a release retarding polymer in tablet formulation has not been reported yet.

In the present work, we have isolated and characterized *Mangifera indica* gum and evaluated its sustained-release properties employing Lornoxicam as a model drug. The matrix tablet of Lornoxicam was formulated using direct compression method and evaluated for appearance, weight variation, hardness, friability, *in vitro* drug release and swelling behavior.

Materials and Methods

Materials

Lornoxicam is procured from Glenmark generics Ltd, Mumbai, India. Mango gum resin was collected from the incised trunk of *Mangifera indica* in Ankola region (Uttar Kannada District). Microcrystalline Cellulose pH 101 (Avicel PH 101) was gift sample from Emcure labs Ltd. Pune. Talc and Magnesium stearate were procured from Apex Chemicals (Ahmedabad, India). All the other solvents, reagents and chemicals used were of either Pharmacopoeial or analytical grade. Different instruments *viz*; Vernier calipers, Monsanto hardness tester, Roche friabilator and disintegration apparatus were supplied by Campbell Electronics, Mumbai. USP XXIII dissolution apparatus-2 was from Tab- Machines, Mumbai, 1601 PC Shimadzu UV Spectrophotometer from Tokyo, Japan and Shimadzu DSC-60, Shimadzu Limited Japan.

Methods

Extraction of *Mangifera Indica* Gum[14-15]

The mango gum resin gum was collected from *Mangifera indica* trees (injured trunk site). It was dried, ground, and passed through sieve no 80. Dried gum (15 g) was stirred in distilled water (300 ml) for 6-8 h at room temperature. The supernatant was obtained by centrifugation. The residue was washed with water and the washings were added to separate supernatant. The procedure was repeated four more times. Finally the supernatant was made up to 500 ml and treated with twice the volume of acetone by continuous stirring. The precipitated material was washed with distilled water and dried at 50-60°C under vacuum. The dried gum was pulverized using a pulverizer and stored in tightly closed container.

Evaluation of Toxicity

Toxicity studies were carried out according to the method of Knudsen and Curtis[16]. The animals used in the toxicity studies were sanctioned by the Institute Animal Ethical Committee (Approval No: KLECP/IAEC/45/2010-11). The male albino rats of Wistar strain weighing 160-200 g were divided into different groups comprising of six animals each. The control group received normal

0.5%CMC solution (20ml/kg i.p). The other groups received 500, 1000, 2000, 3000, 4000 and 5000 mg/kg of MIG suspension in normal saline orally. The animals were observed continuously for the behavioral changes for the first 4 hours and then observed for mortality if any for 72h. Since no mortality, no toxic manifestations were observed and behavioural pattern was unaffected. In chronic toxicity studies, 22 animals were used, divided in to two groups, 6 as control and 16 as test animals. In the test group a dose of 500 mg/kg was administered daily for a period of 30 d. body weights were recorded for both the groups at an interval of 10d. And at the end of 30 days, hematological and biochemical parameters were studied in both the groups and after 30 days of chronic toxicity study the animals were scarified and subjected to histopathological studies.

Physicochemical characterization of mucilage[17-20]

The physicochemical properties such as solubility, swelling index, ash values, loss on drying, precompression parameters and microbial load of the MIG were determined according to official Procedures. The following evaluation parameters were carried out as per the procedures described below.

Solubility

The separated gum was evaluated for solubility in water, acetone, chloroform, methanol, ether and ethanol in accordance with the British Pharmacopoeia specifications.

Determination of swelling index

Swelling characteristics of the separated MIG powder was studied in different media such as 0.1 N hydrochloric acid, pH 7.4 phosphate buffer and distilled water. The swelling index is the volume in ml occupied by 1 g of drug; including any adhering gum after it has been swollen in an aqueous liquid for 4 h. The swelling index of MIG powder was determined according to the British Pharmacopoeia method. 1g of MIG powder was taken in a 25 ml ground glass stoppered cylinder graduated over a height of 120 to 130 mm in 0.5 divisions. To this 25 ml of respective medium was added and this was shaken vigorously every 10 m for 1 h and then allowed to stand for 24 h. The volume occupied by the MIG powder was measured.

The swelling index was computed using the equation

S = V2/V1.

Where; S = Swelling index

V1 = Volume occupied by the gum prior to hydration

V2 = Volume occupied by the gum after to hydration

The test was carried out in triplicate and the average value of swelling index was recorded

Loss on drying

As the inherent moisture in MIG powder/excipients may influence the stability of the tablet dosage form containing moisture sensitive drugs, moisture content of the separated mucilage was detected by loss on drying method. The sample (1 g) was heated at 105^{o}C until constant weight in a hot air oven and percentage loss of moisture on drying was calculated using the formula,

LOD (%) = (weight of water in sample/weight of dry sample)×100.

Total ash

The total ash was determined by placing 3 g of the ground air-dried material in a crucible, spreading the material in an even layer and igniting it by gradually increasing the temperature to 550^{o}C until it is white, indicating the absence of carbon. The crucible was cooled in a desiccator, weighed and the content of total ash in mg per g of air-dried material was calculated.

Acid Insoluble ash

Acid-insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid and igniting the remaining insoluble matter. To the crucible containing the total ash, 25 ml of hydrochloride acid was added, covered with a watch glass and boiled gently for 5 min. The watch glass was rinsed with 5 ml of hot water this liquid was added to the crucible. The insoluble matter on an ash less filter paper was collected and washed with hot water until the filtrate is neutral. The filter paper containing the insoluble matter was transferred to the original crucible, dried on a hot plate and ignited to constant weight. The residue was allowed to cool in a desiccator for 30 min,

weighed without delay and the content of acid insoluble ash in mg per g of air-dried material was calculated.

Microbial load

Microbial count for separated MIG powder was performed as outlined in Indian Pharmacopoeia-1996 for total aerobic microbial count using plate count method. The plate count for bacteria and fungi were measured.

pH determination

This was done by shaking a 1%w/v dispersion of the sample in water for 5 min and the pH determined using a pH meter (Elico, Hyderabad). The data presented here is for triplicate determinations.

Angle of repose

The static angle of repose, a, was measured according to the fixed funnel and free standing cone method. A funnel was clamped with its tip 2 cm above a graph paper placed on a flat horizontal surface. The powders were carefully poured through the funnel until the apex of the cone thus formed just reached the tip of the funnel. The mean diameters of the base of the powder cones were determined and the tangent of the angle of repose calculated using the equation:

Tan a = 2h/D

The data presented here is for triplicate determinations.

Bulk and Tapped densities

2 g quantity each of the powder sample was placed in a 10ml measuring cylinder and the volume, V_0, occupied by each of the samples without tapping was noted. After 100 taps on the table, the occupied volume V_{100} was read. The bulk and tap densities were calculated as the ratio of weight to volume (V_0 and V_{100} respectively). The data presented here is for triplicate determinations.

Hausner's index

This was calculated as the ratio of tapped density to bulk density of the samples.

Compressibility index

This was calculated using the equation:

Compressibility = (Tapped density − bulk density)/Tapped density × 100.

Differential Scanning Calorimetry (DSC) Analysis

Thermal properties of MIG powder were characterized using a Shimadzu DSC-60, Shimadzu Limited Tokyo, Japan. Nitrogen, at the rate of 20 ml/min, was used as purge gas; 2 mg of powdered material were sealed in aluminium pan and heated from 30°C up to 400°C at the rate of 10°C/min, followed by a cooling cycle back to 30°C at the same rate.

Fourier Transform Infra Red (FT-IR) Analysis

The FT-IR spectrum of the sample was recorded in an IR spectrometer (FT–IR: 8101 M, Shimadzu, Japan), using potassium bromide (KBr) discs prepared from powdered samples mixed with dry KBr in the ratio 1:200. Triplicate measurements were made, and the spectrum with the clearest identifiable peaks was chosen.

Phytochemical Examination[21]

Preliminary tests were performed to confirm the nature of gum obtained. The chemical tests that were conducted are: Ruthenium red test, Molisch test, test for reducing sugars and Ninhydrin test.

Characterization of Drug and Excipients

Fourier transform infra red spectroscopy (FTIR)

FTIR spectra of pure Lornoxicam and physical mixture of drug and excipients were recorded on Shimadzu Corporation, (Tokyo, Japan) Model-1601 PC. Potassium bromide pellet method was employed and background spectrum was collected under identical situation. Each spectrum was derived from single average scans collected in the region 650- 4000 cm^{-1} at spectral resolution of $2cm^{-2}$ and ratio against background interferogram. Spectra were analyzed by software supplied by Shimadzu.

Differential Scanning Calorimetry (DSC)

Thermal properties of the pure Lornoxicam and the physical mixture of drug and excipients were analyzed by Shimadzu DSC-60, Shimadzu Limited Japan. The samples were heated in a

hermetically sealed aluminum pans. Heat runs for each sample were set from 30 to 350^0C at a heating rate of 10^0C/ min, using nitrogen as blanket gas.

Formulation of Lornoxicam Sustained Release Matrix Tablet

Sustained release matrix tablets of lornoxicam were prepared by direct compression method as per formula given in Table 1 using different drug: polymer ratios viz. 1:1, 1:1.5,1:2, 1:2.5, 1:3, 1:3.5 for F1, F2, F3, F4, F5, F6, for various batches respectively. MIG was used as matrix forming material, while microcrystalline cellulose was used as filler to maintain the tablet weight. Magnesium stearate and talc used as a lubricant and glidant respectively. All ingredients were passed through a # 60 sieve, weighed, and then thoroughly mixed in a bottle using tumbling method for a period of 15 min. The resultant powder mixture was lubricated with magnesium stearate by further blending for 3 min and finally talc was added to the blend. The resulting powder mixture was subjected to various precompression parameters.

Evaluation of powder Blend

Pre compression parameters

The prepared powder blend was evaluated for various parameters like bulkiness, bulk density, tapped density, angle of repose, compressibility index and Hausner ratio.

Compression of tablet

After evaluation of powder blend the tablets were compressed (8 mm diameter, round flat face punches) using 10 station Rimek tablet compression machine (M/s Karnawati Engg. Ltd. Ahemadabad). A minimum of 50 tablets was prepared for each batch.

Evaluation of tablet

All the tablets were evaluated for following different parameters which includes;

Table 1. Composition of *Mangifera indica* gum Matrix Tablet Formulations

INGREDIENTS (mg)	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**
Lornoxicam	8	8	8	8	8	8
MIG*	8	12	16	20	24	28
Avicel pH101	79	75	71	67	63	59
Magnesium stearate	1	1	1	1	1	1
Talc	4	4	4	4	4	4
Total weight of tablet	100	100	100	100	100	100

*MIG : *Mangifera indica* gum

General appearance

Five tablets from different batches were randomly selected and organoleptic properties such as color, odor, taste, shape, were evaluated.

Hardness

For each formulation, the hardness of five tablets was determined using the Monsanto hardness tester (Cadmach).

Weight Variation

Twenty tablets from each formulation were selected at a random and average weight was determined. Then individual tablets were weighed and was compared with average weight.

Friability

Friability of the tablets was determined using Roche Friabilator. This device subjects the tablets to the combined effect of abrasion and shock in a plastic chamber revolving at 25 rpm and dropping

the tablets at a height of 6 inches in each revolution. Pre weighed sample of tablets was placed in the friabilator and were subjected to100 revolutions. Tablets were de dusted using a soft muslin cloth and re weighed.

The friability (f) is given by the formula.

Friability (f) = 100 (Wo -W) / Wo

Where Wo is weight of the tablets before the test and W is the weight of the tablet after the test

Thickness

Thickness of the tablets was determined using a Vernier caliper. Five tablets from each batch were used, and average values were calculated. It is expressed in mm**.**

Drug content[22]

Ten tablets were weighed and average weight is calculated. All tablets were crushed and powder equivalent to 8 mg drug was dissolved in 8 ml of 0.1N NaOH and the volume was made upto 100 ml with pH 6.8 phosphate buffer. The solution was shaken for 1 h and kept for 24 h. From the stock solution, 1 ml solution was taken in 10 ml volumetric flask and the volume was made with pH 6.8 phosphate buffer. Solution was filtered and absorbance was measured spectrophotometrically (Schimadzu 1601) at 378 nm against pH 6.8 phosphate buffer as a blank. Amount of drug present in one tablet was calculated.

***In vitro* dissolution studies**[23]

The release rate of Lornoxicam from sustained matrix tablets were determined using USP dissolution testing apparatus II (paddle type) at 50 rpm. The dissolution test was performed using 750 ml of 0.1 N HCl (pH 1.2) for 2 h at 37 ± 0.5°C and then 250 ml of 0.2 M tri sodium phosphate ($Na_3PO_4.12H_2O$) was added and pH is adjusted to 6.8 as described in the USP 26/NF monograph. Dissolution test was carried out for a period of 12 h using 0.1N HCl (pH 1.2) for first 2 h and then the pH is adjusted to 6.8 for the rest of the period. The temperature of the dissolution medium is maintained at 37±0.5°C. 10 ml of the sample was withdrawn at regular intervals and replaced with

the same volume pre-warmed with fresh dissolution medium. After filtration, the amount of drug release was determined from the standard calibration curve of pure drug.

Mechanism of drug release

To know the mechanism of drug release from these formulations the data were treated according to first order[24] (log cumulative percentage of drug remaining vs time) higuchi's[25] (cumulative % drug release vs square root of time) and korsmeyer et al's[26] (log cumulative % drug release vs log time) equations along with zero order[27] (cumulative amount drug release vs. time).Korsmeyer and Peppas model was fitted into the following equation.

$Mt / M\mu = K.tn$

Mt /Mμ is the fraction of drug released= the release constant, t= release time, n=diffusion exponent If n =0.89, the release is zero order. If n = 0.45, the release is Fickian diffusion. If $0.45 < n < 0.89$, the release is anomalous diffusion or non fickian diffusion (Swellable & Cylindrical Matrix).

Swelling Studies

The extent of swelling was measured in terms of % weight gain by the tablet. The swelling behavior of all formulation was studied. One tablet from each formulation was kept in a petridish containing pH 6.8 phosphate buffer. At the end of 1 h, the tablet was withdrawn, soaked with tissue paper, and weighed. Then for every 2 h, weights of the tablet were noted, and the process was continued till the end of 24 h. % weight gain by the tablet was calculated by formula;

$S.I = \{(M_t - M_o) / M_o\} X 100,$

Where, S.I = swelling index, M_t = weight of tablet at time's' and M_o = weight of tablet at time t = 0.

Scanning Electron Microscopy

Scanning Electron Microscopy (SEM) of optimized batch (F6) before and different intervals of dissolution were taken. The morphological characters of these scans were compared to hypothesize the mechanism of drug release.

Stability Studies

To assess the drug and formulation stability, stability studies were done according to ICH guidelines[28]. The optimized formulation was subjected to stability study at 40 ±2ºC and 75± 5% RH for 90d. The samples were evaluated for physical changes, hardness, friability, drug content and percentage drug release during the stability studies.

Results and Discussion

In recent years, researchers have become increasingly interested in the utilization of natural biopolymers due to their wide ranging advantages over synthetic polymers. Polysaccharide gums are the materials of choice because they are naturally abundant, biocompatible, biodegradable, and nonimmunogenic.

Gums derived from the plant of *Mangifera indica* was investigated as release retardant for use in sustained release matrix tablet formulations containing lornoxicam.

Physicochemical characterization of *Mangifera indica* gum

The average yield of dried gum obtained from *Mangifera indica* tree was 35% w/w. The gum obtained was an off white to cream yellow color powder, and the viscosity of its 1% aqueous dispersion was 600 cP. The powder was slightly soluble in water and practically insoluble in ether, acetone, chloroform, methanol and ethanol.

The swelling characteristic of MIG was studied in different media; 0.1N hydrochloric acid, phosphate buffer (pH 7.4) and water. The swelling was highest in water (20) followed by 0.1N HCl pH (15) and least in phosphate buffer (10). Generally, the results show that MIG has high swelling index suggesting that the gum may perform well as binder/disintegrant/matrixing agent. The gum is a pH responsive polymer, it is therefore a “smart polymer,” and may find application in controlled release dosage formulations. The moisture content of MIG was low (1.5%), suggesting its suitability in formulations containing moisture sensitive drugs. The total ash, water soluble ash and acid insoluble ash value of MIG was found to be 2.23, 1.3 and 0.4%w/w respectively. Ash values reflect the level of adulteration or handling of the drug. The bulk and tapped densities give an

insight on the packing and arrangement of the particles and the compaction profile of a material. The compressibility index, Hausner ratio and angle of repose of MIG were 16.33%, 0.15 and 22.35° respectively, implying that the MIG has a good flow with moderate compressibility. The loss on drying, ash value and microbial count were well within official limits. The gum obtained from *Mangifera indica* tree was subjected to physicochemical characteristics the results of which are summarized in table 2.

Table 2. Physicochemical characterization of *Mangifera indica* gum

Parameters	**Observation**
Solubility	Slightly soluble in water, practically insoluble in alcohol, chloroform and acetone. Forms thick gel in water.
pH (1% w/v solution)	6.5
Loss on drying	1.5%
Ash value	2.23%
Water soluble ash	1.3%
Acid insoluble ash	0.4%
Sulphated ash	1.03%
Test for foreign matter	Less than 0.1%
Test for arsenic	Less than 1ppm
Swelling ratio	
In water	20.0
In 0.1 N HCl	15.0
In phosphate Buffer 7.4	10.0
True density	1.7g/dl
Bulk density	0.48 g/cc
Tapped density	0.56 g/cc
Compressibity index	16.33%
Hausner ratio	0.15
Angle of repose	22.35
State	Amorphous powder
Odor	No characteristic odor
Taste	Tasteless
Color	Off white- cream yellow color
Total bacterial count	
E.coli	Absent
Salmonella typhi	Absent
S.aureus	Absent
Yield (%)	35
Viscosity (1%)	600 centipoise

Phytochemical screening of *Mangifera indica* gum

Phytochemical tests carried out on MIG confirmed the absence of alkaloids, glycosides and tannins. On treatment of mucilage with ruthenium red, it showed red colour confirming the obtained product as mucilage. A violet ring was formed at the junction of two liquids on reaction with Molisch's reagent indicating the presence of carbohydrates. Mucilage could not reduce Fehling's solution, so the sugars present were non reducing sugars. It reduced Fehling's solution after hydrolysis for 1h with concentrated sulfuric acid under reflux. Mucilage on treating with ninhydrin reagent does not give purple colouration indicating the absence of amino acids. The results of phytochemical screening of MIG are summarized in table 3.

Table 3. Phytochemical screening of *mangifera indica* gum

	Tests	**Observation**
1.	Test for Carbohydrates(Molisch's test)	+
2.	Test for Tannins(Ferric chloride test)	-
3.	Test for proteins (Ninhydrin test)	-
4.	Test for alkaloids (Wagner's test)	-
5.	Test for glycosides(Keller – Killaini test)	-
6.	Test for mucilage (Ruthenium red test)	+
7.	Test for flavonoids (Shinoda test)	-
8.	Test for reducing sugar (Felhing's test)	-
9.	Mounted in 95% alcohol	Transparent angular masses under microscope
10.	Mounting in the iodine	No blue colored particles (starch absent)
11.	Test with cupric –tartaric solution	Red precipitate is produced
12.	Warming with 5M sodium hydroxide	A brown color is produced
13.	Test for chlorides(silver nitrate test)	-
14.	Test for sulphates (barium chloride test)	-

Toxicity study of MIG

To determine the safety level of extracted MIG, acute and chronic toxicity studies were carried out. In acute toxicity study no mortality was observed even at 5000mg/kg of MIG on oral administration and all animals were found to be normal during and at the end of the observation period of three days. Food and water consumption also did not differ significantly and there was no change in general behavior or other physiological activities of the animals in both control and treated groups. To assess the suitability of MIG for the oral delivery we have recorded the body weight profile for the animals during the chronic toxicity studies at regular intervals of 10 days. It was found that the body weight of both control and treatment group and the rate of increase in body weight were comparable. Hence, it could be inferred that chronic administration of the gum might not influence either the food intake or growth. Biochemical and hematological parameters were determined at the end of 30 days of continuous administration of MIG suspension and the biochemical and hematological parameters were found to be comparable to that of normal mice. The results are shown in table 4 and 5 respectively. Histological examination of the main organs like liver, kidney, heart and brain were carried out at the end of 30days of chronic toxicity study. From this study it was revealed that there was no sign of pathological changes in both control and in treatment group.

Table 4. Results of biochemical parameters in rats treated with MIG

Treatment	ALP (U/L)	ACP (U/L)	AST (U/L)	ALT (U/L)	Urea (U/L)	Creatinine (U/L)
Control (0.5%CMC)***	65±4.15*	29±4.25	72±2.34	56±1.25	51±2.10	0.4±0.22
Treatment (MIG)**** 500 mg/kg)	68±4.38**	27±2.02	69±4.10	58±2.87	48±1.65	0.3±0.21

*Data represents as the mean ±SD of 6 animals; **Data represents as the mean ±SD of 16 animals; ***CMC; Carboxy methyl cellulose; **** MIG; *mangifera indica* gum.

Table 5. Results of Hematological changes observed in rats during and after treatment of MIG for 30 days

Treatment	RBC (10^6/mm^3)	WBC (10^3/mm^3)	Hb(g/dl)	N	L	E
Control(0.5% CMC)	4.3±0.05*	7100±0.10	13.58±0.21	8±0.52	85± 0.17	0±0.00
Test(MIG) 500 mg/kg)	4.1±0.07**	6850±0.13	14.12± 0.35	12±0.41	90± 0.21	1± 0.22

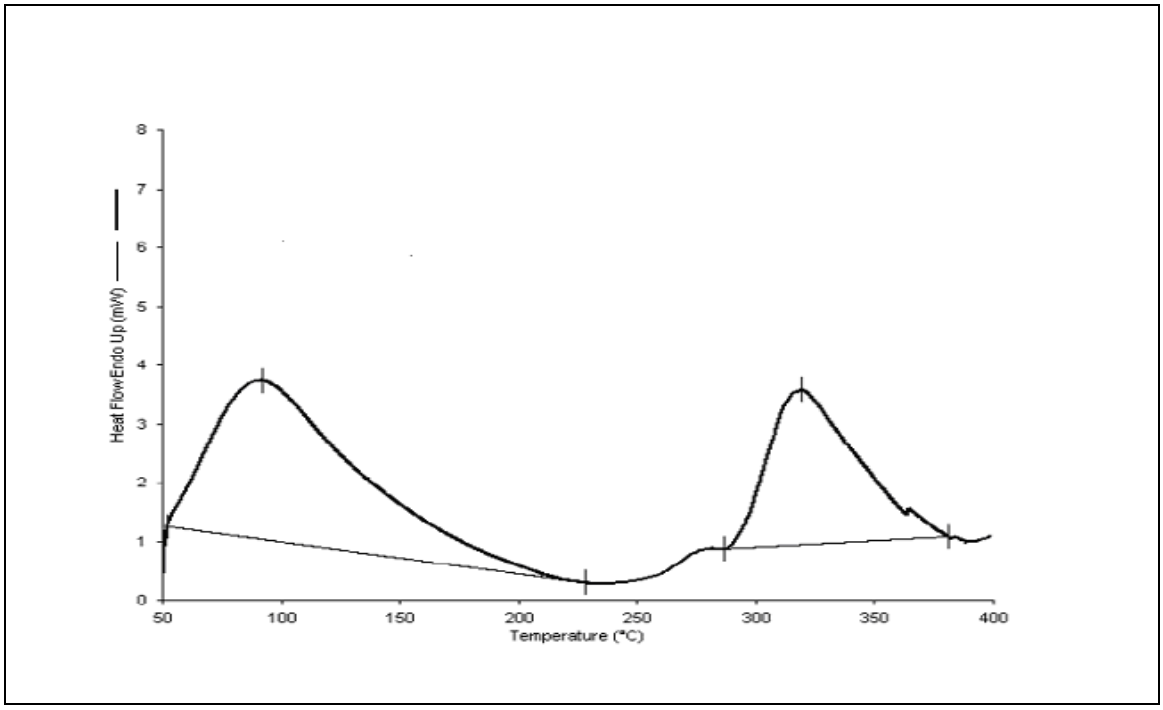
*Data represents as the mean ±SD of 6 animals; **Data represents as the mean ±SD of 16 animals

Characterization of MIG

Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) was used to measure the occurrence of exothermal or endothermal changes with increase in temperature. DSC, because of its sensitivity and accuracy, has been extensively used to study the phase transitions of polymers. The thermogram for MIG is shown in Figure1.It shows that the gum has both amorphous and crystalline portions. Glass transition (Tg) temperature occurred at 94°C while a melting peak was observed at about 320°C.

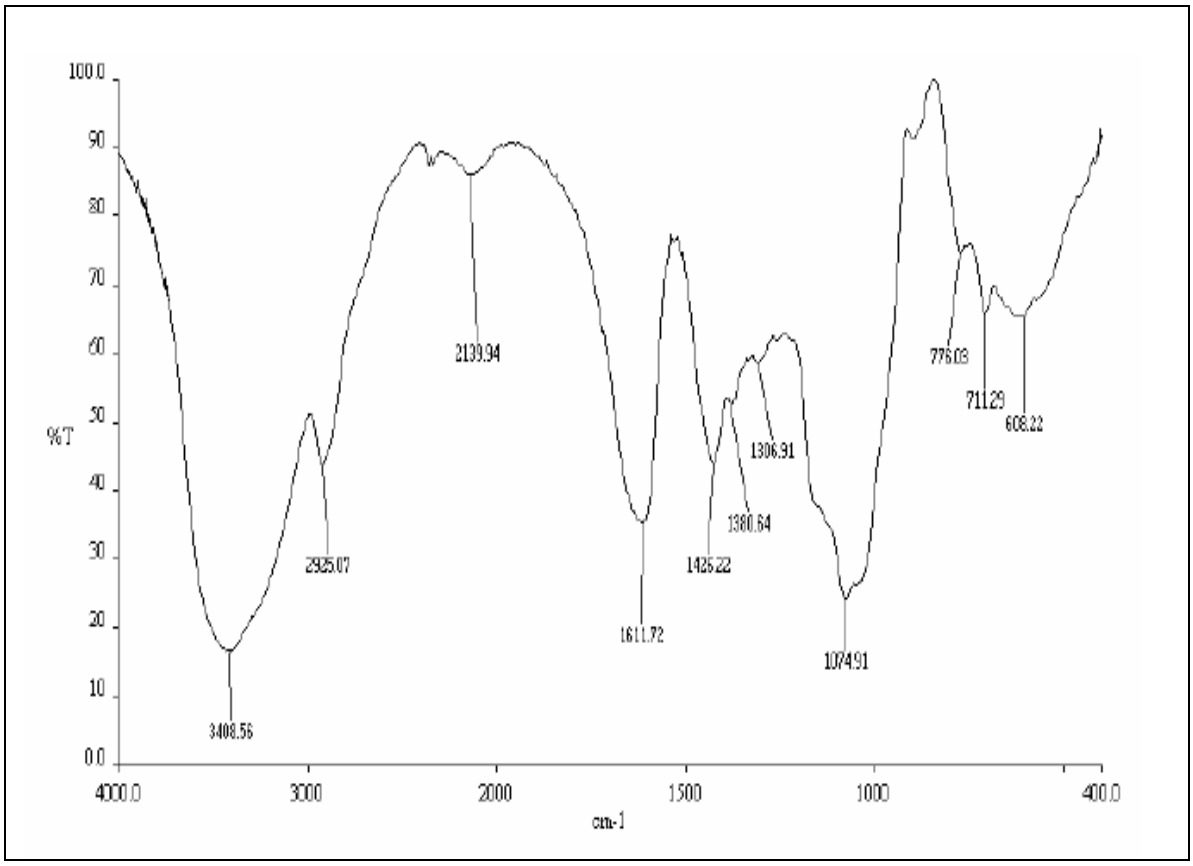
Fig.1. Differential scanning calorimetry curve of *mangifera indica* gum Powder



Fourier Transform Infra Red (FT-IR)

The IR spectrum of MIG is shown in Figure 2. The finger print region of the spectrum consists of two characteristic peaks between 700 and 1316 per cm, attributed to the C-O bond stretching. The band at 1604 per cm was assigned to the O-H bending of water. There are absorptions (weak) in the 1730 per cm area that indicate carbonyls. The absence of significant aromatic stretches in the 1660-1690 per cm region and the weakness of the stretches, imply that there is a modest amount of peptidic cross linking by amide bond formation. The sharp band at 2939 per cm is characteristic of methyl C-H stretching associated with aromatic rings. The broad band at 3286 cm^{-1} is due to the hydrogen-bonding that contributes to the complex irrational stretches associated with free inter and intra-molecular bound hydroxyl groups which make up the gross structure of carbohydrates.

Fig.2. FTIR spectrum of *mangifera indica* gum powder



Characterization of drug and excipients

The formulation additives in concentrations used did not affect the stability and Ultraviolet absorbance of the drug.

Fourier transform infra red spectroscopy (FTIR)

The interaction study between the drug and excipients in different formulations were performed using FTIR spectrophotometer. The pellets were prepared on KBr press. The spectra were recorded over the wave number range of 4000 to 650 cm^{-1}. The FTIR spectrum of lornoxicam showed a characteristic peak at 3,090 cm^{-1} corresponding to NH stretching vibration. Intense absorption peak was found at 1,642 cm^{-1} due to the stretching vibration of the C=O group in the primary amide. Other peaks were observed at 1,597 and 1,559 cm^{-1} and were assigned to bending vibrations of the N–H group in the secondary amide. The stretching vibrations of the O=S=O group appeared at 1,157, 1,387, and 1,336 cm^{-1}. Other prominent peaks appeared at 827.94 cm^{-1} corresponding to CH aromatic ring bending and heteroaromatics and at 766.8 cm^{-1} due to the C–C_l bending vibration, which indicates groups is match with structure of drug and confirm the purity of the drug. FTIR-spectra of drug and its physical mixture with excipients are exactly same, and there is no shift of peaks or disappearance of principle peaks or modification of the principle peaks indicating that there is no interaction between the drug and excipients. FT-IR spectrum of pure drug and its physical mixture is represented in Figure 3 and Figure 4.

Differential Scanning Calorimentry (DSC)

Any possible drug polymer interaction can be studied by thermal analysis. The DSC thermogram of lornoxicam was typical of a crystalline substance, exhibiting a sharp exothermic peak at 232.9°C corresponding to its melting and decomposition. The thermograms of the physical mixtures of lornoxicam with other excipients (1:1) showed the existence of the drug exothermic peak which could indicate the absence of interaction between lornoxicam and other excipients. The DSC thermogram of pure drug and its physical mixture is represented in Figure 5.

Fig.3. Infrared Spectrum of pure lornoxicam

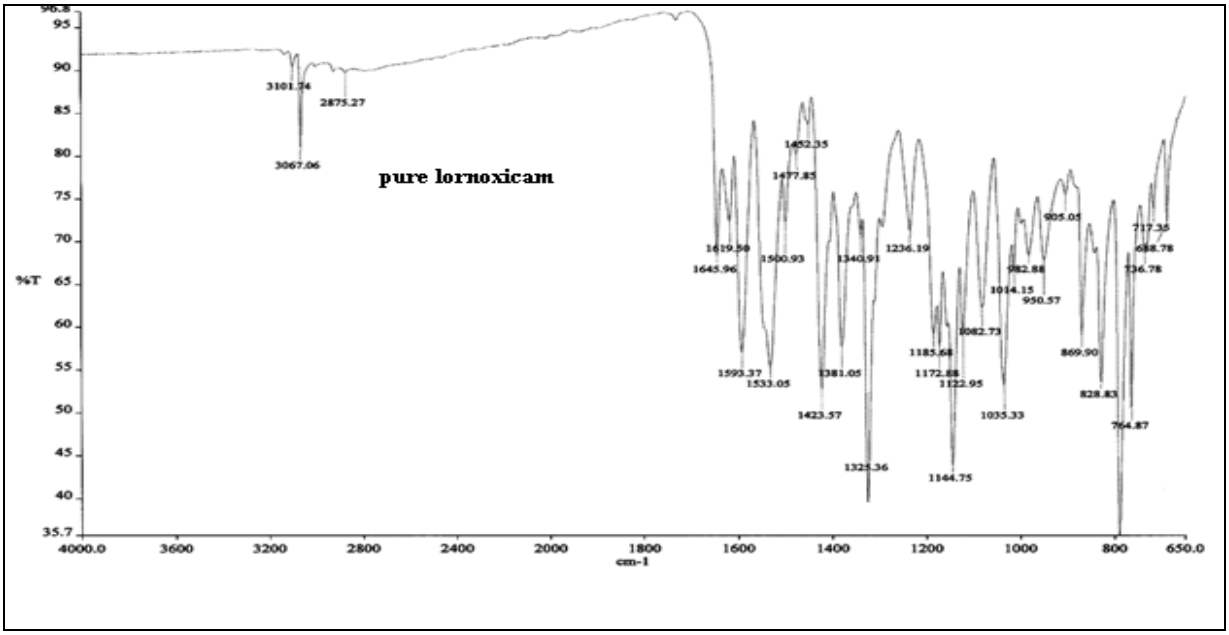


Fig.4. Infrared Spectrum of physical mixture of lornoxicam and excipients

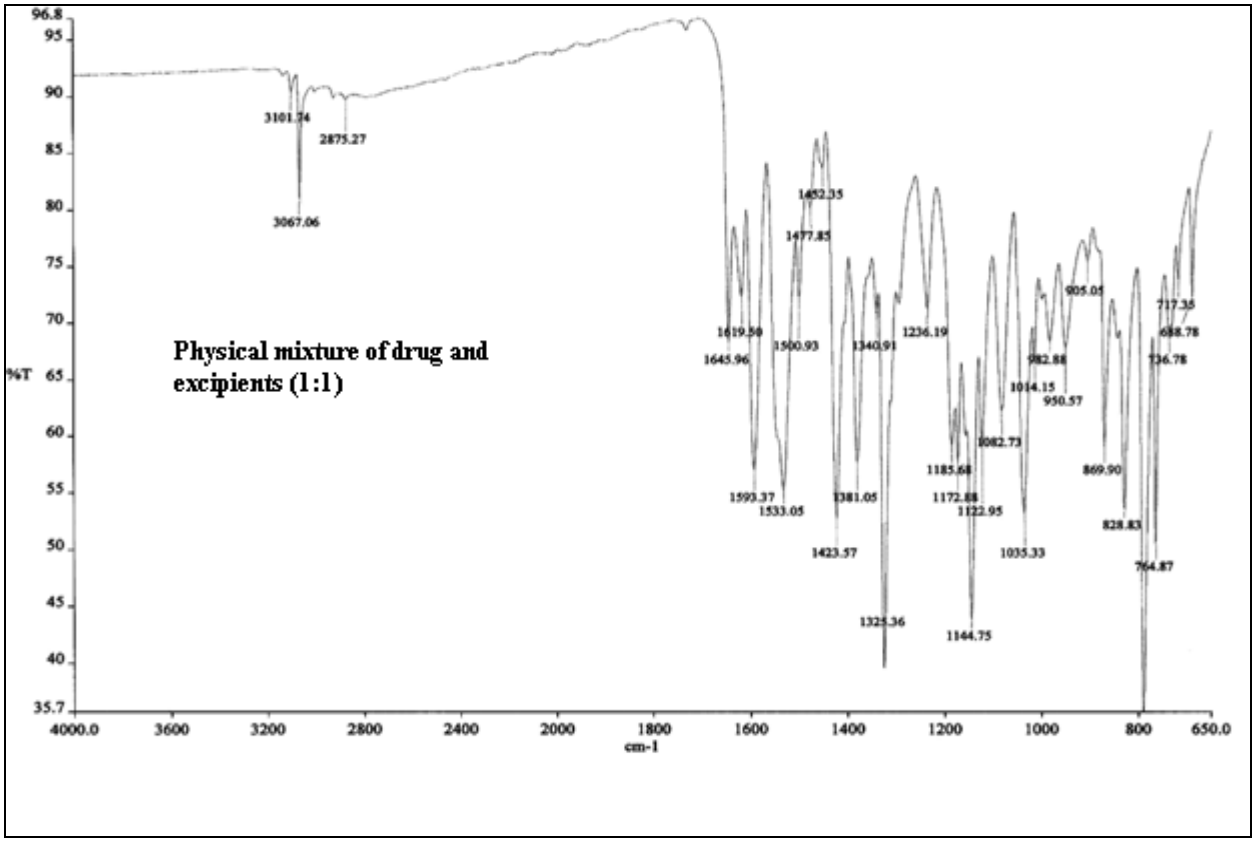
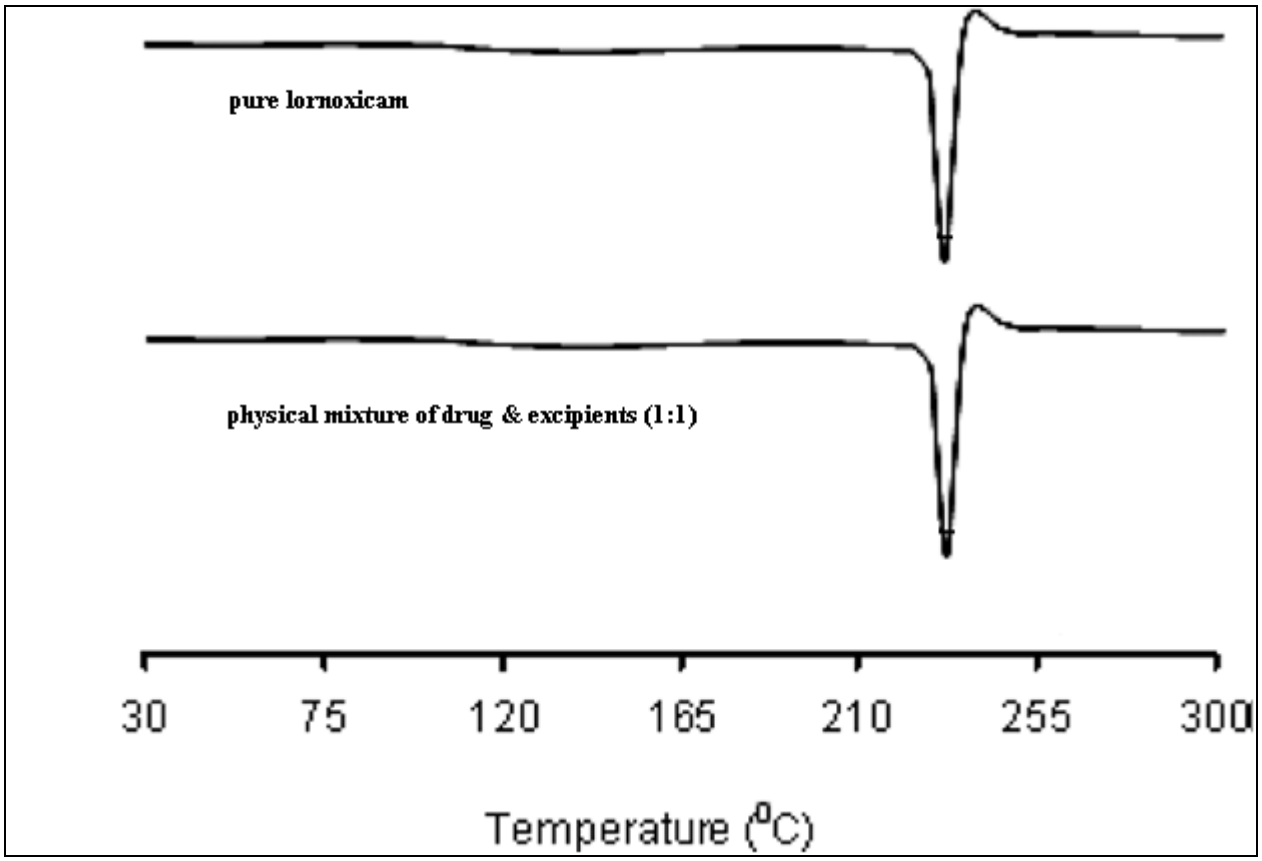


Fig.5. DSC Thermogram of physical mixture of lornoxicam and excipients



Results of flow properties of lornoxicam powder blend

Powder blend prepared for compression of matrix tablets were evaluated for their flow properties like angle of repose, bulk density, tapped density, Hausner ratio and compressibility index. The results were shown in Tables 6. Angle of repose was in the range of 26.65 ±0.02 to 29.3 ± 0.02. The bulk density of the granules was in the range of 0.57 ± 0.02 to 0.62 ±0.06 gm/ml; the tapped density was in the range of 0.65±0.06 to 0.72 ± 0.01 gm/ml, which indicates that the granules were not bulky. The Compressibility index and Hauser ratio was found to be in the range of 12.31 to 16.18 and 1.14 to 1.19 respectively.

Table 6. Results of Flow properties of Lornoxicam powder blend

Formulation code	Angle of repose(θ)	Bulk density (g/cm^3)	Tapped density (g/cm^3)	Carr's index (%)	Hausner ratio (H_R)	Flowability
F1	29.32±0.10	0.57±0.04	0.68±0.01	16.18	1.19	good
F2	29.73±0.16	0.59±0.02	0.69±0.05	14.52	1.17	good
F3	28.20±0.05	0.57±0.02	0.65±0.06	12.31	1.14	good
F4	29.30±0.02	0.58±0.05	0.66±0.02	12.12	1.14	good
F5	26.65±0.02	0.62±0.06	0.72±0.01	13.89	1.16	good
F6	28.20±0.05	0.57±0.02	0.65±0.06	12.31	1.14	good

*All values are expressed as mean ± SD, n=3

Results of physical properties of lornoxicam sustained release matrix tablets

The results of physical properties of lornoxicam sustained release matrix tablets are shown in Tables 7. The thickness of matrix tablets was measured by vernier caliper and was ranged between 2.82±0.12 to 3.48±0.07 mm. The hardness of the matrix tablets was measured by Monsanto tester and was controlled between 6.5±1.45 to 8.15±1.20 kg/cm^2. The friability was below 1% for all the formulations. Weight variations for different formulations were found to be 98±0.03 to 102±0.05mg. The percentage of drug content for F1 to F6 was found to be in between 99.5% to 101.2% of lornoxicam it complies with official specifications. Thus all the physical attributes of the prepared tablets were found be practically within control. The lornoxicam matrix tablets were off-white, smooth, and flat shaped in appearance.

Table 7. Results of Physical properties of Lornoxicam matrix tablets

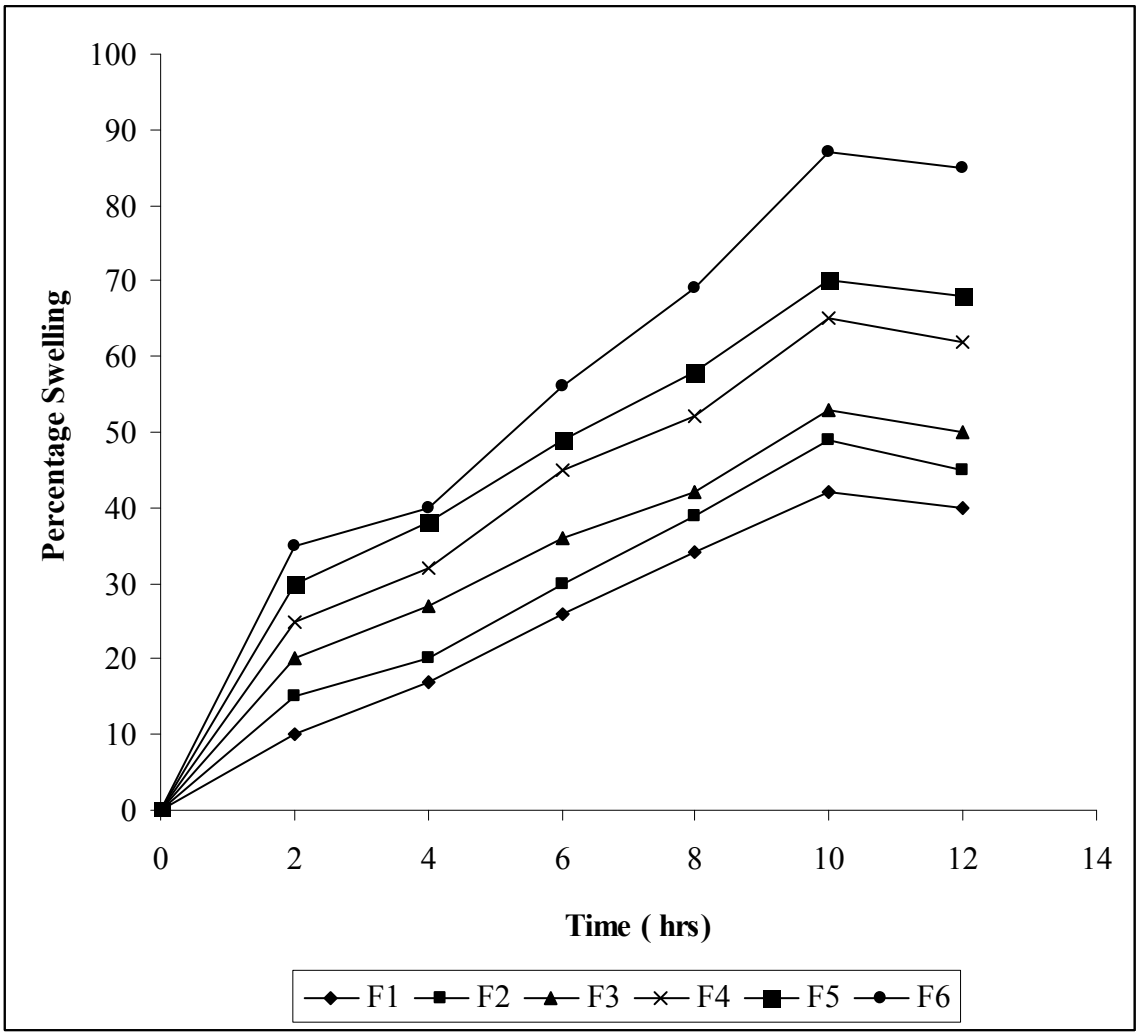
Formulation code	Thickness (mm) (n=5)	Hardness (kg/cm^2) (n=5)	Friability (%) (n=5)	Drug content (%) (n=10)	Weight variation (mg) (n=20)
F1	3.16±0.06	7.50±1.25	0.50±0.02	100.2±3.9	100±0.02
F2	2.88±0.10	8.10±1.40	0.85±0.05	101.2±5.2	98±0.03
F3	3.05±0.05	6.80±1.35	0.44±0.03	99.5±2.5	101±0.01

F4	3.48±0.07	6.50±1.45	0.62±0.06	99.9±2.1	102±0.05
F5	2.85±0.03	7.40±1.30	0.73±0.07	100.5±3.6	100±0.01
F6	2.82±0.12	8.15±1.20	0.80±0.01	101.2±5.0	98±0.05

Swelling behavior of matrix tablets

The extent of swelling was measured in terms of % weight gain by the tablet. The swelling behavior of all formulation was studied. Figure 6 showed the swelling characteristics of MIG containing tablets. The swelling index was calculated with respect to time. As time increases, the swelling index was increased, because weight gain by tablet was increased proportionally with rate of hydration up to certain limit. Later on, it decreases gradually due to dissolution of outermost gelled layer of tablet into dissolution medium. Similar results were earlier reported for mucilage of *Hibiscus rosasinensis*; matrix tablets formulated using pure mucilage showed greater swelling and lesser erosion as compared with the matrix tablets containing mucilage and drug[29]. The release of drug from hydrophilic matrices occurs as a result of complex interaction between diffusion, dissolution, and erosion mechanisms. On coming in contact with water, hydrophilic matrices undergo gel formation, and progressive phase transition from glassy to rubbery state takes place. This results in solvation of individual polymer chains. As the swelling continues, the swollen matrix retains more water until the shear forces in the dissolution medium disentangle the individual polymer chains from the matrix. The direct relationship was observed between swelling index and polymer concentration, and as polymer concentration increases, swelling index was increased.

Fig.6. Results of swelling behaviour of different batches of matrix tablets.

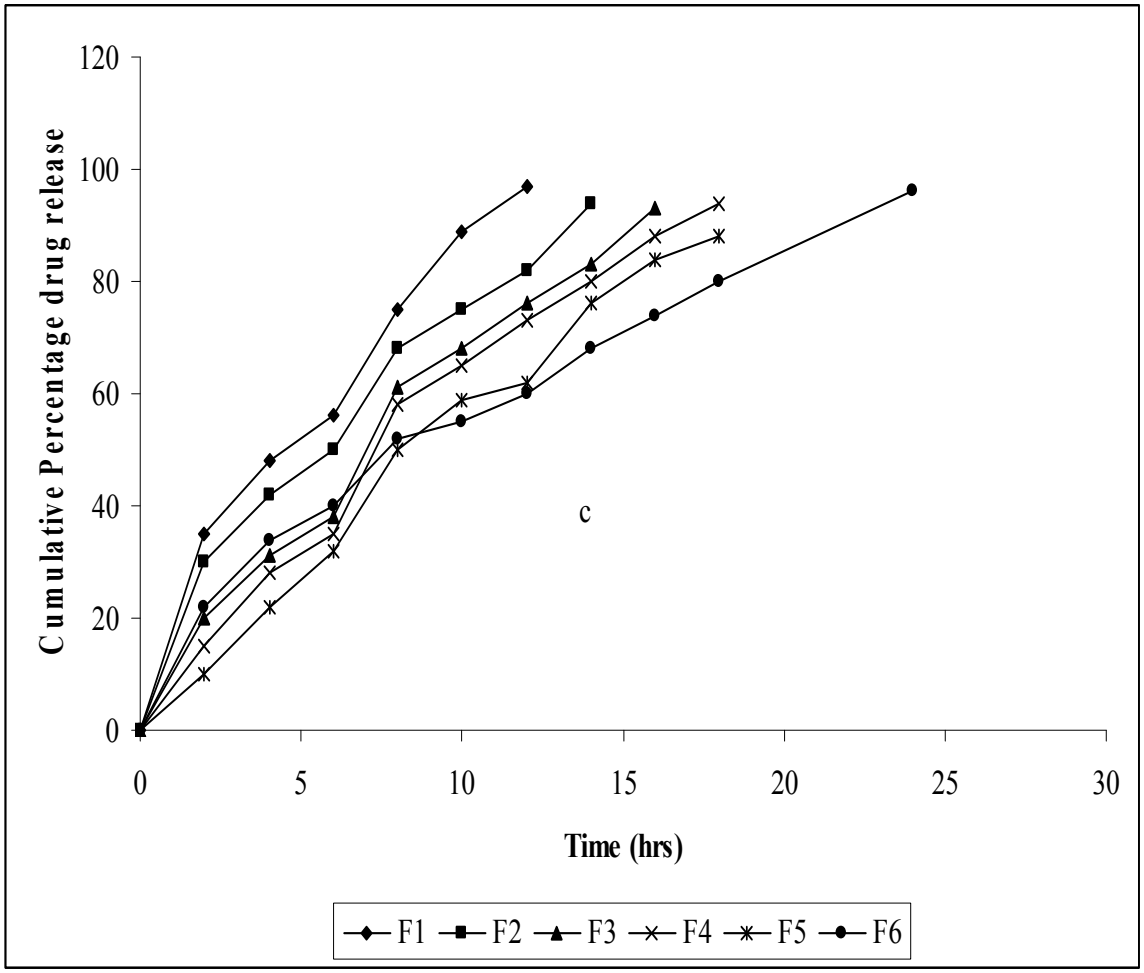


Results of *In vitro* drug release study

The release rate pattern of lornoxicam from various batches of formulated tablets is illustrated in Figure 7. The results show that <1% of the drug dissolved during the first 2 h in 0.1 N HCl. It can be observed from the results that, as the proportion of gum in tablets was increased from the drug to gum ratio of 1:1 (F1) to 1:3.5 (F6), there was a decrease in the release rate, and release of lornoxicam was extended with 98% and 82% of the lornoxicam getting released from the tablets of batches F1 and F6, respectively. Earlier studies have also reported the decrease in the release rate of drug with increase in the proportion of matrix polymer[30-31]. The decrease in the release rate of drug from the matrix tablets with increase in the gum proportion can be attributed to increase in the gel

strength and to the formation of gel layer with longer path of diffusion, resulting in reduction of diffusion coefficient of the drug. The tablets at drug to gum ratio of 1:3.5 (F6), shown more optimum result as release modifier. The drug releases from the tablets decreases with the increases in gum concentration.

Fig.7. Results of *In vitro* dissolution profile of different batches of matrix tablets

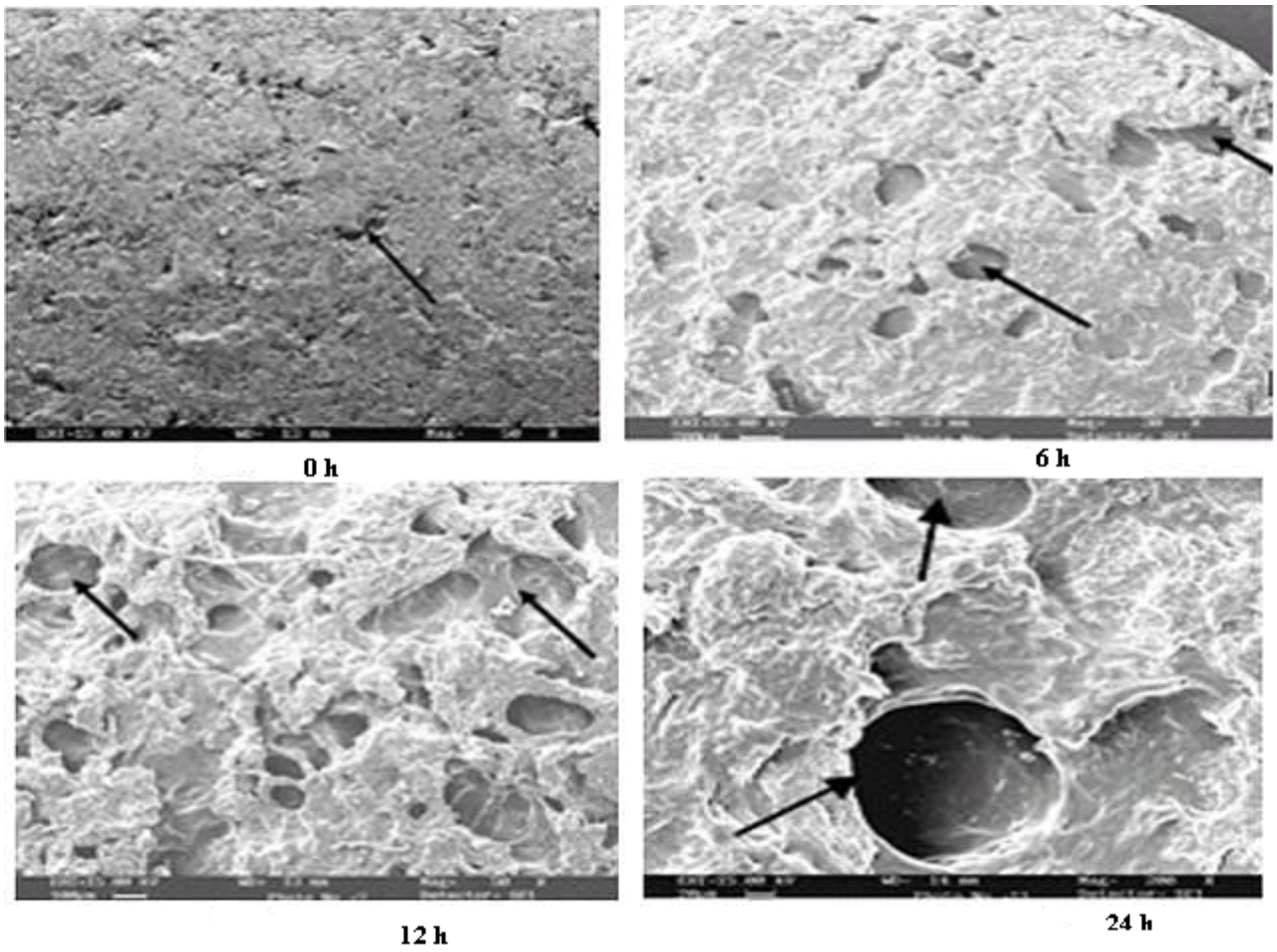


Results of Scanning Electron Microscopy

Figure 8 displays the scanning electron photomicrographs of tablets of optimized batch F6. The photomicrographs show the surface morphology of tablets after 0 and 24 h of dissolution study. The surface of tablets of batch F6 after 24-h study shows the presence of both gelling structures and pores on the surface. Thus, the presence of both pores and gelling structure indicates the

combination of diffusion and erosion mechanism in the release of lornoxicam from the matrix tablet of batch F6.

Fig.8. Surface morphology of optimized matrix tablet formulation (F6) at different time intervals



Mechanism of drug release

To determine the mechanism of drug release, the release rate of optimized formulation (F6) was fitted into various kinetic models. Figure 9-12 shows the result of modeling and drug release kinetics of F6 lornoxicam matrix tablet. It can be observed from the results that the release rate data of F6 batch of lornoxicam matrix tablets formulated using MIG as the matrix fitted to the Higuchi's square root release kinetics, as indicated by highest value of r^2. Further, the results of n, the release

exponent of Korsmeyer–Peppas equation, show that the lornoxicam is released by a combination of diffusion and erosion mechanisms ($0.45 < n < 0.89$) in the case of batch F6 ($n = 0.581$).

Fig.9. Zero order release kinetics of optimized formulation (F6)

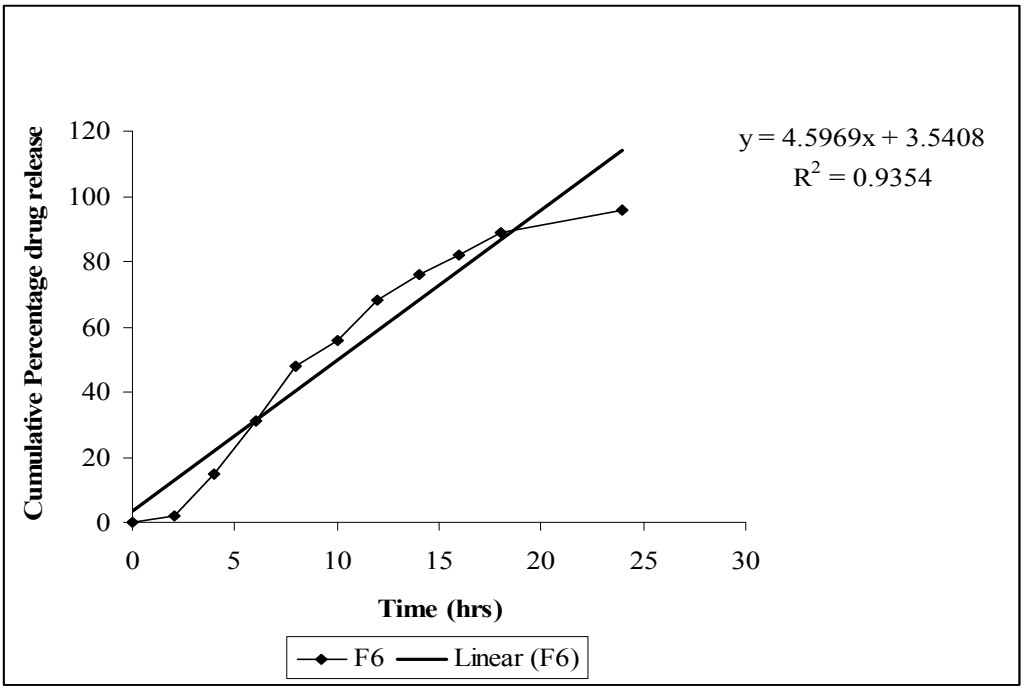


Fig.10. Higuchi matrix release kinetics of optimized formulation (F6)

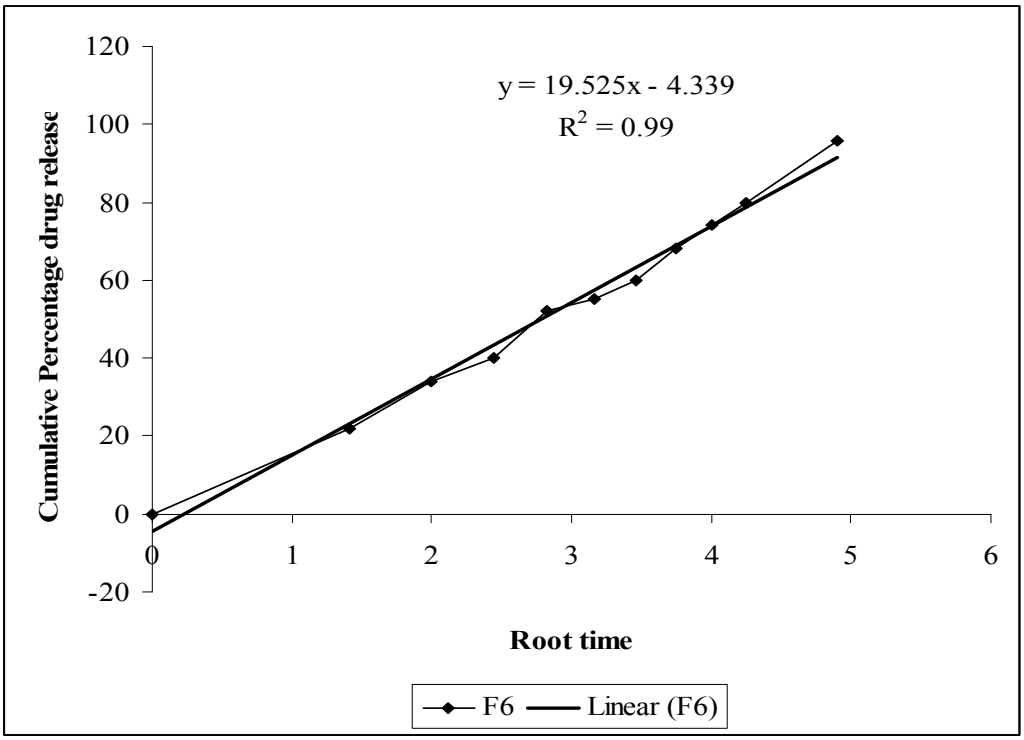


Fig.11. Korsmeyer and Peppas release kinetics of optimized formulation (F6)

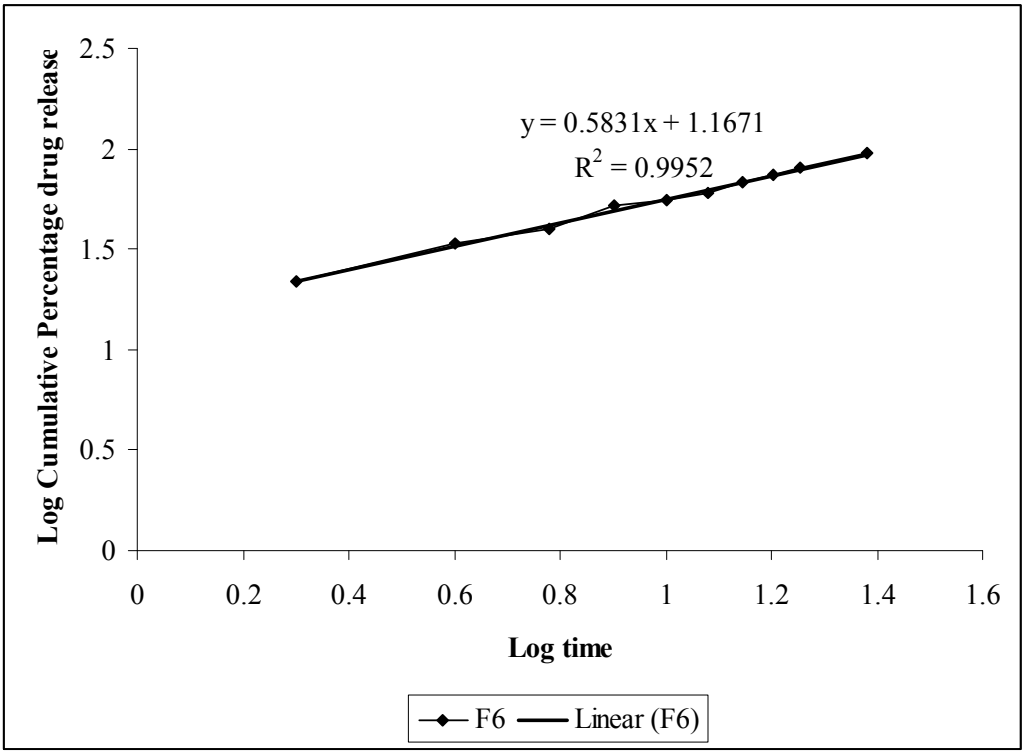
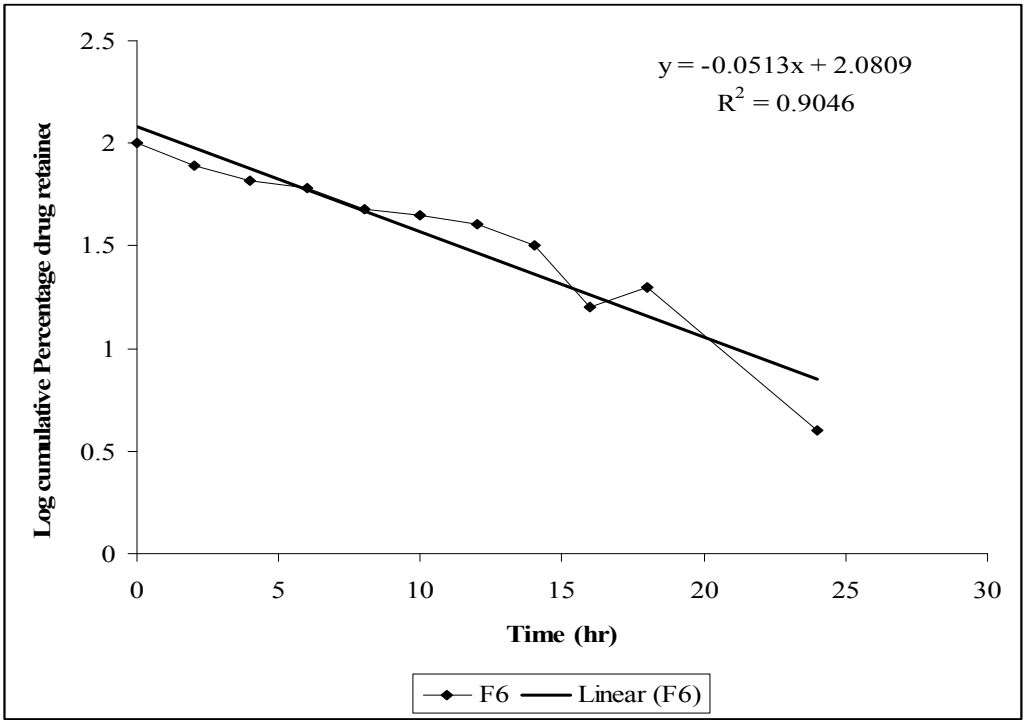


Fig.12. First order release kinetics of optimized formulation (F6)



Results of Stability study

The optimized formulation F6 was kept at accelerated (40±2 °/75±5% RH) storage conditions for a period of 3 months. After stability test period, tablets were analyzed for drug content, hardness, friability and *in vitro* release. Stability studies result showed that there was no significant change in hardness, friability, drug content, and dissolution profile of formulation F6. The formulation was stable under accelerated conditions of temperature and humidity.

Conclusion

The present study provided an insight into the evaluation of MIG as a release retardant in the formulation of sustained release matrix tablets because of its good swelling, good flow and suitability for matrix formulations. The results of the present study demonstrated that MIG sustained the drug release. Drug release from MIG matrix formulations was dependent on the mucilage to drug ratio. As the concentration of MIG increased, drug release is retarded due to increase in the gel strength and to the formation of gel layer with longer path of diffusion, resulting in reduction in diffusion coefficient of the drug. The matrix was found to release the drug following Higuchi square root kinetics. The formulations containing lower proportion of MIG (1:1 to 1:3.5) released the drug by combination of diffusion through the matrix and matrix erosion and through the swollen matrix. Formulation containing MIG in the proportion of drug to polymer ratio of 1:3.5(F6). So, it can be concluded that MIG can be used as matrix-forming agent for sustained drug delivery in tablet formulations.

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